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New Cephalosporins Cefotaxime, Cefpimizole, BMY 28142, and HR 810 in Experimental Pneumococcal Meningitis in Rabbits

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Four new cephalosporins, cefotaxime, cefpimizole (U 63196E), BMY 28142, and HR 810 were evaluated in experimental pneumococcal meningitis. Cefotaxime penetrated only moderately into the cerebrospinal fluid of rabbits with meningitis, whereas cefpimizole, BMY 28142, and HR 810 all exhibited unusually good penetration. The bactericidal activity in infected cerebrospinal fluid was comparable for the four drugs.

The high morbidity and mortality of bacterial meningitis and the emergence of pathogens resistant to conventional antibiotics prompt a constant search for new and more effective drugs for the treatment of meningitis. Besides being nontoxic, candidate drugs should penetrate well to the site of infection (5) and exhibit rapid bactericidal activity against the infecting microorganism (5, 8). Since penetration into the cerebrospinal fluid (CSF) can only be assessed in vivo and since in vitro tests do not reliably predict the bactericidal activity of antibiotics in meningitis (9), animal models provide an excellent opportunity to evaluate new antibiotics for the treatment of meningitis. We used a well-standardized rabbit model of pneumococcal meningitis to assess the CSF penetration and bactericidal activity of four recently developed broad-spectrum cephalosporins: cefotaxime; cefpimizole (U 63196E), [(7-beta-D-(−)-alpha-{4(5)-carboxyimidazole-5(4)-carboxamide}-phenylacetamido-3-(4-beta-sulfoethylpyridinium)-methyl-3-cephem-4-carboxylic acid sodium salt]); BMY 28142, [7-{alpha-(2-aminothiazol-4-yl)-alpha-(z)-methoximinoacetamido}-3-(1-methylpyrrolidinio)-methyl-3- cephem-4-carboxylate]; and HR 810, an aminothiazolyl-alpha-methoxyimino cephalosporin.

MATERIALS AND METHODS

Cefotaxime and HR 810 were provided by Hoechst-Roussel Pharmaceuticals (Somerville, N.J.), BMY 28142 is an experimental drug from Bristol Laboratories (Syracuse, N.Y.), and cefpimizole was provided by The Upjohn Co. (Kalamazoo, Mich.).

A clinical isolate of *Streptococcus pneumoniae* type 3 was used for all experiments. Suspensions of the organism were aliquoted and stored at −70°C. The sample was thawed and diluted in saline to give a final inoculum of 5 log CFU. The MICs of the four antibiotics were determined by macrobroth dilution in tryptic soy broth with 5% sheep erythrocytes in a final volume of 1.0 ml (10). The MBC was defined as the lowest concentration showing 99.9% killing of the organism after streaking 0.01 ml from each tube onto a blood agar plate and incubating overnight at 37°C in 5% CO_2.

Pneumococcal meningitis was induced in 79 New Zealand white rabbits, 2 to 2.5 kg, by previously described methods (1). In brief, a dental acrylic helmet was attached to the skull of the animals under anesthesia (30 mg of pentobarbital per kg intravenously). This helmet permitted placement of the animals in a stereotactic frame and injection of the inoculum (5 log CFU in 0.3 ml of saline) directly into the cisterna magna. A long-lasting anesthesia with urethane (Sigma Chemical Co., St. Louis, Mo.), 2 g/kg intravenously, was induced 18 h after infection, at which time all animals showed signs of meningitis (rectal temperature >39.6°C, CSF pleocytosis, and CSF bacterial counts of 4 to 7 log CFU/ml). Untreated, the disease is fatal in 100% of the animals, usually within 24 to 76 h. Animals were placed in the stereotactic frames, and the antibiotics were infused over the next 7 h at a constant rate through a peripheral vein. Individual animals received only one drug at one concentration.

The treatment regimens ranged as follows: cefotaxime, 2 to 150 mg/kg per h; cefpimizole, 1 to 75 mg/kg per h; BMY 28142, 0.065 to 25 mg/kg per h; and HR 810, 0.08 to 10 mg/kg per h. These were chosen to achieve CSF concentrations that varied over a broad spectrum. The infusion was started with a loading dose of 10% of the total drug administered, which resulted in constant serum levels during the treatment period. The CSF was collected through a spinal needle in the cisterna magna, and blood was simultaneously collected through a femoral artery line at 0, 1, 3, 5, and 7 h. CSF bacterial titers were determined by quantitative cultures of 10-fold dilutions onto blood agar plates. The remainder of the CSF and serum samples was stored at −70°C for determination of antibiotic concentrations (within 1 week of the experiment).

Drug concentrations in serum and CSF were determined by an agar diffusion bioassay (11) with *Pasteurella multocida* (no. UC802; The Upjohn Co.) as the test organism for cefpimizole and *Escherichia coli* ATCC 10536 as the test organism for cefotaxime, BMY 28142, and HR 810.

The percent drug penetration into CSF was calculated as the ratio of CSF concentration to serum concentration multiplied by 100. Statistical analysis for comparison of bactericidal rates was done on unpaired data by Students *t* test. Least-square regression line analysis was performed to assess correlations between CSF drug concentrations and rates of killing.

RESULTS

Table 1 shows the mean serum and CSF antibiotic concentrations and the percent penetration into the CSF for the different agents. All four drugs showed increasing penetration into the CSF during the 7-h treatment (cefotaxime, 1.5%

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[1 h] to 4.6% [7 h]; cefpimizole, 6.9% [1 h] to 27.6% [7 h]; BMY 28142, 7.3% [1 h] to 31.7% [7 h]; and HR 810, 7.4% [1 h] to 21.3% [7 h]). Cefotaxime penetrated the infected CSF to a limited extent, with a mean (± standard deviation) of 3.5 ± 2.7% of simultaneous serum levels. In contrast, cefpimizole, BMY 28142, and HR 810 all penetrated well into the CSF of infected animals with mean percent penetrations of 16.5 ± 7.8% for cefpimizole, 20.2 ± 10.20% for BMY 28142, and 21.8 ± 6.4% for HR 810.

Whereas cefotaxime, BMY 28142, and HR 810 were all highly active in vitro, with MBCs of 0.03 μg/ml, cefpimizole (MBC, 1.0 μg/ml) showed only moderate activity against the infecting *S. pneumoniae*.

When the bactericidal activity was examined in the CSF of infected rabbits in relation to the CSF concentrations achieved, all four cephalosporins showed comparable activity. For each of the four drugs, the mean bactericidal rate (expressed as reduction in CSF bacterial titers per hour) was higher at CSF drug concentrations in excess of 10-fold the MBC than at CSF concentrations less than 10 times the MBC (Table 2). This difference was statistically significant, except for HR 810, which approached maximal bactericidal rates at lower CSF concentrations (relative to the MBC) than the other drugs. When tested by the least-square method, HR 810 as well as the other three study drugs showed a significant correlation between CSF drug levels and the rate of bacterial killing ($r = 0.53$ and $P < 0.05$ for HR 810). For all four drugs examined, only CSF concentrations in excess of 10 times the MBC consistently produced bactericidal rates >0.4 log CFU/ml per h.

DISCUSSION

Our study of four new cephalosporins in experimental pneumococcal meningitis shows moderate differences among the four drugs examined. Cefotaxime did not penetrate as well into the CSF of rabbits with pneumococcal meningitis as did the three other drugs. The low percent penetration of cefotaxime (3.5%) was comparable to that of penicillin (2.8%), cephalothin (0.7%), cefamandole (2.5%), and cefazolin (3.1%) in this animal model (6, 9). Ceftriaxone (5.8%), cefoperazone (6.4%), cephacetrile (7.6%), cefuroxime (8.7%), moxalactam (11.5%), and ampicillin (12.1%) achieve increasingly higher percent penetrations (2, 3, 6, 7, 9), whereas cefpimizole (16.5%), BMY 28142 (20.2%), and HR 810 (21.8%) all exhibited excellent CSF penetrations. The ability of an antibiotic to gain access to the site of infection is considered critical in the therapy of meningitis (4, 5). Since high CSF concentrations of an antibiotic relative to its MBC are needed to exhibit maximal bactericidal activity in CSF (5, 9), the good CSF penetration of drugs like cefpimizole, BMY 28142, or HR 810 may represent an advantage, particularly for the treatment of meningitis caused by only moderately susceptible microorganisms. Although one drug (cefpimizole) was not very active against the infecting organism in vitro, the in vivo activities of the four drugs were comparable when adjusted for their relative CSF concentrations. As reported recently (9) and confirmed in this study, a positive correlation between CSF drug concentrations and bactericidal activity in CSF appears to be a universal characteristic of beta-lactam antibiotics. To achieve maximal activity in CSF, drug concentrations must exceed the MBC by at least a factor of 10 to 30. Maximal bactericidal rates even at very high CSF concentrations rarely exceeded 1 log CFU/ml per h in the present study as well as in our recently published study (9). Thus, as judged by their bactericidal activity in CSF, the four drugs examined in this study all appear promising as potential drugs for the treatment of bacterial meningitis caused by susceptible organisms. In addition, cefpimizole, BMY 28142, and HR 810 have the advantage of penetrating exceptionally well into infected CSF.

TABLE 1. Concentration of cefotaxime, BMY 28142, HR 810, and cefpimizole in serum and CSF of rabbits with pneumococcal meningitis

Drug	Dosage[a] (*n*)	Serum[b] (μg/ml)	CSF[b] (μg/ml)	% Penetration[c]
Cefotaxime	75.0 (3)	160.4 ± 33.55	6.1 ± 2.07	5.7 ± 0.61
	50.0 (6)	49.5 ± 12.29	2.3 ± 1.36	4.3 ± 2.31
	5.0 (6)	8.7 ± 2.98	0.34 ± 0.46	3.4 ± 4.14
	2.0 (3)	4.5 ± 0.4	0.07 ± 0.02	1.6 ± 0.42
Cefpimizole	75.0 (5)	179.6 ± 64.3	31.3 ± 20.92	17.2 ± 7.08
	25.0 (5)	116.8 ± 13.76	11.6 ± 2.85	10.6 ± 2.43
	2.5 (6)	9.3 ± 5.06	1.5 ± 0.57	18.1 ± 8.71
	2.0 (3)	8.2 ± 0.31	1.8 ± 0.99	22.6 ± 12.21
	1.0 (2)	2.9 ± 1.12	0.54 ± 0.53	16.2 ± 12.3
BMY 28142	25.0 (6)	57.9 ± 13.18	9.6 ± 5.09	16.2 ± 5.98
	4.0 (6)	9.0 ± 2.31	2.1 ± 1.35	22.6 ± 14.56
	0.4 (6)	1.6 ± 0.43	0.35 ± 0.14	22.0 ± 9.02
	0.065 (6)	0.16 ± 0.10	0.04 ± 0.02	27.2 ± 12.0
HR 810	10.0 (5)	32.0 ± 8.95	6.4 ± 3.24	19.3 ± 4.33
	0.5 (6)	1.0 ± 0.33	0.24 ± 0.07	24.8 ± 7.53
	0.08 (5)	0.23 ± 0.09	0.02 ± 0.005	9.3 ± 3.23

[a] Milligrams per kilogram per hour.
[b] Mean ± standard deviation.
[c] ([CSF]/[Serum]) × 100; mean ± standard deviation.

TABLE 2. Bactericidal activity of cefotaxime, cefpimizole, BMY 28142, and HR 810 in CSF of rabbits with pneumococcal meningitis

Drug	Rate of bacterial killing (change in log CFU/ml per h) with CSF concn of:		*P*[a]
	<10 × MBC	≥10 × MBC	
Cefotaxime	−0.44 ± 0.14 (8)[b]	−0.84 ± 0.18 (10)	0.001
Cefpimizole	−0.45 ± 0.23 (14)	−0.69 ± 0.22 (7)	0.02
BMY 28142	−0.44 ± 0.03 (6)	−0.74 ± 0.22 (17)	0.001
HR810	−0.60 ± 0.26 (9)	−0.76 ± 0.09 (7)	0.1

[a] By the two-tailed Student *t* test.
[b] Numbers within parentheses indicate the numbers of animals examined.

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